Molecular Ecology Laboratory

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Laboratory Protocol

Protocol number: 4
Protocol description: DNeasy DNA extraction from sloughed skin samples
Original reference: Qiagen and Qbiogene tissue extraction protocols
Original entry: Carrie LeDuc
Last updated: 23 October 23, 2003
Updated by: Carrie LeDuc

Required materials:
Fastprep Lysing Matris A tubes

Weigh boats

Razor Blades

Squirt Bottle of mQH_2O

Forceps

DNeasy tissue kit reagents

100% EtOH

Required equipment:
Fastprep homogenizer

Water bath at 55^0 C

Heat block at 70^0 C

Timer

Centrifuge

Vortex

Procedure:

1. Turn on the water bath to 55^0 C and heat block to 70^0 C.

2a. If there is a large amount of sloughed skin remove a small piece of tissue from vial with tweezers and wash by soaking for a few seconds in mQ water in a clean weigh boat. Do not squirt the tissue with water. A razor blade may be necessary to cut a small piece off the sloughed skin. Use the tweezers to remove the tissue from the water and put it into a 1.7ml tube.

2b. If there is only a small amount of sloughed skin and it is not possible to remove the skin from the DMSO then the skin can be filtered out using filter paper in a funnel on top of an Erlenmeyer flask. Pour the entire DMSO/tissue solution slowly into the filter. If there is still tissue in the vial rinse the vial with mQ water and pour the rinse through the filter. Wash the DMSO out of the filter paper with more mQ water until there is no salt left and the tissue is near the bottom of the filter. Remove the filter and use a clean razor blade and tweezers to collect the tissue off the filter. Put the tissue into a 1.7ml tube.

3. To the 1.7ml tube add:
   a. Tissue
   b. 180ul Buffer ATL
   c. 20ul PK (from DNeasy extraction kit)
4. Vortex.

Note: It is not necessary to Fastprep sloughed skin.

5. Incubate tubes at 55^0 C for 1 hr (or 37^0 C overnight) in the water bath. Vortex occasionally.
6. Add 200ul Buffer AL to the Fastprep tube and vortex.
7. Incubate tubes at 70^0 C for 10min on the heat block.
8. Add 200ul 100% EtOH to the Fastprep tubes and vortex.
9. Quick spin the Fastprep tube and pipet supernatant from the Fastprep tube into a DNeasy column.
10. Centrifuge at 8000rpm for 1min.
11. Discard collection tube containing flow-through and place column in a new collection tube.
12. Add 500ul Buffer AW1 to the column.
13. Centrifuge at 8000rpm for 1min.
14. Discard collection tube containing flow-through and place column in a new collection tube.
15. Add 500ul Buffer AW2 to the column.
16. Centrifuge at 13000rpm for 3min.
17. Discard collection tube containing flow-through and place column in a 1.7ml tube.

18. Add 100ul Buffer AE to the column and allow to sit for 1min.
19. Centrifuge at 8000rpm for 1min to elute DNA.
20. Repeat steps 21-22 with the same 100ul of Buffer AE.